

What is claimed is:

1. An apparatus for total internal reflection microscopy of a sample, comprising  
a microscope objective lens;  
an excitation beam path for passing light through the objective lens to said sample; and  
a coupling element arranged in a back focal plane of the objective lens or in a plane which is conjugate to said back focal plane;  
said coupling element comprising a first area for relaying light to the objective lens for total internal reflection illumination of said sample and a second area; wherein said second area is capable of separating light emitted by said sample and passing through said excitation beam path in reverse direction from said excitation beam path or said second area is capable of relaying light into an illumination path for epi-illumination of said sample or said second area is capable of both separating light emitted by said sample and passing through said excitation beam path in reverse direction from said excitation beam path and relaying light an illumination path for epi-illumination of said sample; wherein said second area is spatially separate from said first area and does not overlap with said first area; and  
wherein a distance between said optical axis of the objective lens and that boundary of said first area which is nearer to said optical axis of the objective lens is selected such that the light beams passing from said first area into the objective lens are imaged by the objective lens at angles onto said sample for which total reflection of these light beams occurs.
2. The apparatus according to claim 1, wherein the light for total internal reflection illumination of said sample is laser light.
3. The apparatus according to claim 2, wherein said first area is transparent for said laser light.
4. The apparatus according to claim 3, wherein said second area is reflective for light emitted from said sample or wherein said second area is reflective for light for epi-illumination of said sample.

5. The apparatus according to claim 4, wherein said first area is an aperture in a reflecting disc.
6. The apparatus according to claim 2, wherein said first area is reflective for laser light for total internal reflection illumination of said sample.
7. The apparatus according to claim 6, wherein said coupling element is transparent for laser light for total internal reflection illumination of said sample in a region outside said first area.
8. The apparatus according to claim 6, wherein said coupling element in regions outside said first area is transparent for light emitted by said sample or wherein said coupling element in regions outside said first area is transparent for light for epi-illumination of said sample.
9. The apparatus according to claim 1, wherein said coupling element is tilted relative to said back focal plane of the objective lens or relative to said plane conjugated relative to said back focal plane.
10. The apparatus according to claim 1, wherein said first area is formed as a first portion having a strip-like shape and extending radially relative to said optical axis of the objective lens.
11. The apparatus according to claim 10, wherein said coupling element comprises a second portion which is arranged point-symmetric regarding said first portion relative to said optical axis of the objective lens for transmitting or reflecting laser light which has been passed onto said sample via said first portion and which has been totally reflected at said sample.
12. The apparatus according to claim 11, wherein said coupling element is tilted around an axis passing through the center of said first portion and said second portion and is tilted relative to said back focal plane of the objective lens or relative to said plane conjugated to said back focal plane.
13. The apparatus according to claim 1, wherein said coupling element has the shape of a circular or elliptical disc.

14. The apparatus according to claim 13, wherein said first area of said coupling element radially extends over distances only which correspond to a numerical aperture of more than 1.35 in said conjugated focal plane of said microscope objective lens.
15. The apparatus according to claim 1, wherein the center of said coupling element has a region for illuminating the objective lens with total internal reflection illumination laser light for adjusting said apparatus.
16. An apparatus for total internal reflection microscopy of a sample, comprising  
a microscope objective lens;  
an excitation beam path for passing light through the objective lens to said sample; and  
a coupling element arranged in a back focal plane of the objective lens or in a plane which is conjugate relative to said back focal plane;  
said coupling element comprising a first area which is non-transparent for light passing to the objective lens for total internal reflection illumination of said sample,  
wherein a distance between said optical axis of the objective lens and a periphery of said first area is selected such that light beams passing around periphery of said first area into the objective lens are imaged by the objective lens at angles onto said sample for which total reflection of these light beams occurs; and  
wherein said first area is capable of separating light emitted by said sample and passing through said excitation beam path in reverse direction from said excitation beam path or wherein said second area is capable of relaying light into the objective lens for epi-illumination of said sample;
17. The apparatus according to claim 16, wherein said first area is reflective for light emitted by said sample or wherein said first area is reflective for light for epi-illumination of said sample.
18. The apparatus according to claim 17, wherein said first area consists of a first surface which faces the light beams for total internal reflection illumination of said sample and which is non-transparent for these light beams and of a second surface which is arranged relative to said first surface at an angle and which is reflective for light

- emitted from said sample or which is reflective for light for epi-illumination of said sample.
19. The apparatus according to claim 18, wherein said first surface is a reflective circular surface which is oriented perpendicular to the light beams for total internal reflection illumination of said sample.
  20. The apparatus according to claim 19, wherein said second surface is a sectional surface of a plane at an oblique angle with a real or imaginary cone having a base surface which forms said first surface.
  21. the apparatus according to claim 16, wherein a light source for total internal reflection illumination of said sample is a non-coherent light source.
  22. The apparatus according to claim 21, wherein said first area has a shape of an annular ring.
  23. The apparatus according to claim 1, wherein light beams for total internal reflection illumination of the sample reaching said coupling element and light beams for epi-illumination of said sample reaching said coupling element are oriented at an angle of about 90°.
  24. The apparatus according to claim 1, wherein a means for determining the intensity of the light for total internal reflection illumination of said sample, a means for determining the intensity of the light totally reflected by said sample and a control means are provided, wherein said control means is capable of maintaining the intensity of the light for total internal reflection illumination of said sample below a predetermined threshold intensity if a ratio between the intensity of the light for total internal reflection illumination of said sample and the intensity of the light totally reflected at said sample exceeds a predetermined threshold ratio.
  25. The apparatus according to claim 24, wherein an optical element is arranged in said excitation beam path which is capable of reflecting a portion of incident light beams for total internal reflection illumination of said sample to said means for determining the intensity of the light for total internal reflection illumination of said sample, said optical element being capable of transmitting the remainder of the incident light beams for TIR illumination of said sample to said first area of said coupling element.

26. The apparatus according to claim 24, wherein the material, the thickness and the angle of said optical element relative to said optical axis is selected such that a wavelength-dependent beam displacement in the plane of said first area of said coupling element is achieved which compensates at the sample the wavelength-dependence of the penetration depth of the evanescent field of the incident light beams for total internal reflection illumination of said sample.
27. The apparatus according to claim 1, wherein said apparatus is capable of performing fluorescence observation of said sample.